Separation of pepsins in human gastric juice: analysis of proteolytic and mucolytic activity

DAVID A. HUTTON,* ADRIAN ALLEN,** JEFFREY P. PEARSON,* RICHARD WARD, and CHRISTOPHER W. VENABLES;

Departments of *Physiological Sciences and **Surgery, Medical School, University, Framlington Place, Newcastle upon Tyne NE2 4HH, U.K.

Pepsin and acid in gastric juice are the endogenous aggressors in peptic ulceration. The gastroduodenal mucus barrier with an epithelial bicarbonate secretion forms the first line of defense against acid (surface neutralization) and pepsin (permeability barrier) (Allen & Garner, 1980). This mucus barrier consists of a thin but continuous layer of water-insoluble gel adherent to the mucosa; the thickness of the gastric mucus layer in man is 50–450 µm, median 180 µm. Pepsin acts as a mucolytic agent by degrading the polymeric structure of the gel-forming glycoprotein and thus dissolves the mucus (Scawen & Allen, 1977). In vivo, luminal pepsin, while not able to diffuse through the mucus cover to the epithelium, will dissolve the gel at its luminal aspect and this loss must be balanced by secretion of new mucus to maintain a barrier (Allen et al., 1984).

The presence of seven pepsins in human gastric juice has been demonstrated by agar gel electrophoresis and the relative amounts of pepsins 1–7 in order of decreasing electrophoretic mobility (Etherington & Taylor, 1967). Increased levels of pepsin 1, the most acidic pepsin, have been associated with peptic ulcer disease (Walker & Taylor, 1980). Here improved methods for the measurement of total pepsin activity and the relative amounts of pepsins 1, 3 and 5 are described, together with a method for measuring mucus degrading activity in samples of human gastric juice.

Total pepsin activity in human gastric juice was measured by a modification of the method of Lin et al. (1969), using succinyl-albumin as substrate and porcine pepsin A as standard. Enzyme (0.075–1.0 µg, 0.2 ml) and substrate (0.5 ml, 6 mg/ml) were incubated for 30 min at 37 °C and pH 2.2. Enzyme activity was assayed by the addition of 0.5 ml of 0.05% (w/v) sodium bicarbonate followed by 0.5 ml of 0.05% (w/v) trinitrobenzenesulphonic acid to trinitrophenylate the free amino groups formed. Colour was developed by incubation at 50 °C for 10 min followed by addition of 0.5 ml of 10% (w/v) SDS and 0.25 ml of 1 M-HCl to complete the reaction. Absorbance at 340 nm was measured. The above assay was about 10 times more sensitive (down to ~0.1 µg compared with ~1.0 µg of pepsin) than the standard method for pepsin in gastric juice (modifications of Anson & Mirsky, 1932) which depends on the selective precipitation of undigested protein.

The relative amounts of the different pepsin types in human gastric juice have previously been estimated semi-quantitatively by visual assessment of gels negatively stained for pepsin activity after electrophoretic separation (Etherington & Taylor, 1980). We have now adapted the gel electrophoretic method for the quantification of pepsins 1, 3 and 5 in gastric juice. After electrophoresis at pH 5.3 (Etherington & Taylor, 1967), a small strip of the gel was stained for location of pepsins and the rest sectioned into 0.5 cm strips and enzyme present eluted with 0.01 M-HCl at 4 °C (15 h). Pepsin activity at pH 2.2 in each gel fraction was determined using the assay above. The amount of pepsin I in pentagastrin stimulated gastric juice from duodenal ulcer patients was found to be significantly greater (15.2% of total pepsin ± 1.52, n = 9) than levels in gastric juice from non-symptomatic volunteers (8.7% of total pepsin ± 0.7, n = 7).

The mucus-degrading activity of pepsin can be assayed by measuring the fall in viscosity of a solution of polymeric pig gastric mucus glycoprotein (5 mg/ml) over 30 min at 37 °C on incubation with the gastric juice (0.5%), w/w, enzyme/substrate). Loss of viscosity is due to enzymic breakdown of the polymeric structure of the glycoprotein (demonstrated by gel filtration on Sepharose 2B; Mantle & Allen, 1978). Pentagastrin stimulated gastric juice from duodenal ulcer patients exhibited greater mucolytic activity than that from non-symptomatic volunteers at the optimum pH (2.2) and particularly between pH 3.0 and 5.0. For example, at the same pepsin concentration (measured by proteolytic digestion of serum albumin), gastric juice from duodenal ulcer patients had approximately four times more mucus degrading activity at pH 4.0 than that from non-symptomatic controls.

Our results concur with previous studies reporting elevated levels of pepsin I in patients with duodenal ulcers (Walker & Taylor, 1980). Purified pepsin 1 has been shown to hydrolyse mucus glycoprotein two times and six times more than pepsin 3 (the major pepsin) at pH 2.2 and 4.0 respectively (Pearson et al., 1984, 1986). Pentagastrin stimulated gastric juice from duodenal ulcer patients exhibited substantial mucolytic activity between pH 2.0 and 5.0, similar to pepsin 1, while gastric juice from non-symptomatic volunteers had little activity above pH 4.0, similar to pepsin 3. These studies suggest that increased mucolytic activity associated with raised pepsin 1 levels may contribute to enhanced degradation of mucus and a consequent weakening of the mucus barrier shown to occur in peptic ulcer patients (Younan et al., 1982).

Evidence for a 60 000 M, protein in adult human hyaline cartilage

S. C. MALIK, P. D. PICKFORD and J. P. PEARSON

Department of Physiological Sciences, The Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH, U.K.

The extracellular matrix of hyaline cartilage consists mainly of collagen and proteoglycans but it also contains non-collagenous matrix proteins/glycoproteins (Paulsson & Heinegård, 1984). Little is known about the function of these molecules yet they make up about 5% of the dry weight of cartilage. Some have been shown to interact with proteoglycan aggregates (Paulsson & Heinegård, 1979; Pearson & Mason, 1979). Here we report the presence of a non-collagenous protein, M, about 60 000, bound strongly to the proteoglycan aggregate fraction from human hyaline cartilage.

Human costal cartilage was obtained at post mortem within 8 h of death; menisci and acetabulum cartilages were obtained at operation and stored at -70°C until used. The perichondrium and any areas of extensive calcification were removed. The tissue was then shredded with a surform blade and extracted at 4°C for 24 h in 0.4 M guanidine hydrochloride (GuHCl). After 30 min digestion at 37°C, most of the 60 000 band had disappeared, the large link protein (48 000) had gone, and a strongly staining band had appeared at 43 000; also, there was evidence of low-M, material on the gels. After 60 min there was no evidence of a band at 60 000. These data show the susceptibility of the 60 000 protein to proteolysis.

The Figure shows 7.5% SDS gels run in reducing conditions. Lane 1, human costal cartilage A, was digested with Clostripain (Caputo et al., 1980); the results are shown in Fig. 1. Before digestion the 60 000 protein was clearly visible on the gels. After 30 min digestion at 37°C, most of the 60 000 band had disappeared, the large link protein (48 000) had gone, and a strongly staining band had appeared at 43 000; also, there was evidence of low-M, material on the gels. After 60 min there was no evidence of a band at 60 000. These data show the susceptibility of the 60 000 protein to proteolysis.

The band produced at 43 000 is probably composed of the small link protein produced from the larger by proteolysis and possibly some enzyme (M, between 40 000 and 50 000). The above data suggest that this 60 000 protein is not a small proteoglycan or the hyaluronic acid-binding region because it does not appear to be glycosylated and it is susceptible to proteolysis. At present no role has been found for this protein; however, the lack of aggregation in adult human hyaline cartilages and the presence of this protein as the major protein component of the A, fraction could mean it is involved in limiting aggregation.

Abbreviation used: GuHCl, guanidine hydrochloride.

Fig. 1. Clostripain digestion of human costal cartilage A, fraction
The Figure shows 7.5% SDS gels run in reducing conditions. Lane 1, human costal cartilage A, was digested with Clostripain. Lane 2, A, was loaded on to the gels. Lane 3, A, 30 min (5 units of enzyme/ml, 6 mg of A, /ml); lane 4, A, 60 min; lane 4, standards. Two hundred microlitres of sample was loaded on to the gels.